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Production of spike and nucleocapsid recombinant proteins of porcine epidemic diarrhea virus for antibody detection by ELISA

Anchalee Srijangwad [a], *Dachrit Nilubol* [a], *Wanchai Chongcharoen* [b], *Waranyoo Phoolcharoen* [c], *Taksina Chuanasa* [c], *Angkana Tantituvanon* [b,*]

[a] *Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand*

[b] *Department of Pharmaceutics and Industrial Pharmacy, Chulalongkorn University, Bangkok, Thailand*

[c] *Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand*

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Porcine epidemic diarrhea (PED), a devastating enteric disease in pigs, is caused by PEDvirus (PEDV) [1]. Reduced severity of clinical diseases was reported to associate with neutralizing antibody titers in colostrum. However, viral neutralization assay (VN) is laborious and not suitable for routine diagnosis. Spike protein plays an important role in stimulating neutralizing antibody that might be suitable for PEDV diagnosis. The objectives of this study were to produce spike (S) and nucleocapsid (N) recombinant proteins (rS and rN) specific for PEDV and to develop ELISA assay to detect neutralizing antibody against PEDV in colostrum and serum.

Spike (S) and nucleocapsid (N) genes were amplified by PCR from PEDV isolated from infected pig. PCR products were ligated to vector, cut with restriction enzymes and ligated to expression vector. The recombinant pG-S and pG-N plasmids were transformed into *Escherichia coli*, followed by sequencing analysis and optimized the condition for protein expression. Recombinant proteins were detected by using western blotting analysis. rS and rN were purified. ELISA plates were coated with rS and rN and antibody detection of PEDV both IgG (rS-IgG and rN-IgG) and IgA (rS-IgA and rN-IgA) from sera and colostrum of pigs in comparison to neutralizing assay (NA).

Molecular weights of rS and rN were 42 and 78 kDa, respectively. Average of optical density between SN positive and SN negative group founded all of test positive group was significantly higher than negative group ($P < 0.0001$) except IgA could not be detected in these sera. ELISA test of rS-IgG in sera founded sensitivity (62%), specificity (98%), overall agreement

* *E-mail address:* tuvanont@gmail.com.

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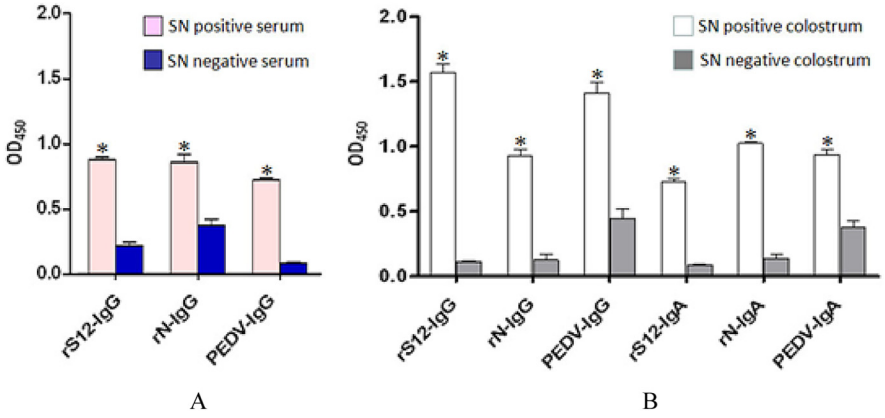
Fig. 1 – ELISA test of S and N recombinant proteins from 100 pig sera (A) and 100 pig colostrum (B).



A B

(80%) and Kappa (K = 0.6) higher than rN-IgG. In contrast with colostrum founded sensitivity (78%), overall agreement (88%) and K (0.77) of rN-IgG higher than rS-IgG but rS-IgA was highly of sensitivity (90%), specificity (100%), overall agreement (95%) and almost perfect agreement (K = 0.9). The rS and rN could be very useful for the development of ELISA test kit.

This study demonstrated that S and N recombinant proteins can be produced. Statistical analysis showed highly significant especially ELISA coating with rS for IgG and IgA detection from pig colostrum. Interestingly, rS of IgA detection in colostrum highly correlated with SN test. However, the development still needs to be tested in more samples, and then develop ELISA test kit for PEDV detection in pig farm.

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